DRAFT

BRIEF COMMENTS

on

Question 3 (see below) from the Institute of Medicine Committee to Assess the Science Base for Harm Reduction from Tobacco

Question 3:

"What is the effect of removing a component or components of tobacco on the qualitative and quantitative composition of the new species of smoke? What is the empirical evidence that such alteration would be associated with reduced or altered disease incidence in humans?"

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E-mail of April 17, 2000

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1 General Remarks

In the strictest sense, the question should apply to components of the native tobacco only. However, "tobacco" is used in these comments as a synonym for the total filler of cigarettes, therefore the following comments cover

- flavors and other ingredients added to the tobacco
- components of processed tobacco (e.g., sheet material made of milled stems)
- natural material in or on native tobacco.

The phrase "qualitative and quantitative composition of the ... smoke" in the question is interpreted in the following comments as referring to the

- biological activity (e.g., mutagenic activity in the Ames Assay) and the
- yield or concentration of the numerous constituents in the smoke.

These comments do not cover two other approaches, that have been shown to alter cigarette smoke:

- selective filtration of the smoke in order to remove defined smoke components
- variation of the burning process, e.g., in electrically heated cigarettes, that has been shown to result in a biologically less active cigarette smoke (Reininghaus et al., 1998)

2 Chemical Composition and Biological Activity of Cigarette Smoke after Renunciation/Removal of Filler Components

2.1 Flavors and other ingredients

During cigarette production flavors are added to the tobacco to enhance or to modify the natural taste of the smoke. Humectants are applied to the tobacco as processing aids and as a way to retain a certain amount of tobacco moisture during the shelf life of the cigarettes. These ingredients generally have Food and Drug Administration and/or Flavor and Extract Manufacturers GRAS-status, i.e., they are "Generally Recognized As Safe" for use in food. However, there is a concern that the addition of these ingredients, particularly the non-volatile ones, may give rise to combustion or pyrosynthesis products with unwanted biological activity (Pauly, 1999).
In order to determine the potential effects of ingredients on smoke chemistry and biological activity of the smoke, cigarettes with and without the addition of ingredients have been investigated.

When 333 ingredients (for technical reasons split into three groups) were added to American-blend cigarettes at an approximate use level and at a multiple of the normal use level, an extensive chemical analysis of the resulting smoke indicated essentially no major changes. There were only isolated increases and decreases in constituents when compared to the control cigarette without the addition of ingredients. These changes, while statistically significant, were not supported by any biological changes. The

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mutagenicity as measured in the Salmonella Reverse Mutation (Ames) Assay was not increased nor was the cytotoxicity as measured in the Neutral Red Uptake Assay with mouse embryo BALB/c 3T3 cells. Also the inhalation toxicity determined in a 90-day inhalation study in rats (OECD protocol 413) with special emphasis on irritative changes in the respiratory tract was not increased. A few ~~increases and decreases~~ changes (not) in some of the numerous endpoints were all well within the normal variation of such bioassays and considered to be due to statistical inference (Roemer et. al., 2000).

These data corroborate the findings of another rat inhalation study where over 170 ingredients were added in groups to 6 test cigarettes. The smoke from these cigarette types showed no increase in the incidence of histopathological findings in the respiratory tract or the occurrence of new findings there not already present in the groups exposed to smoke from the control cigarette without the addition of ingredients (Gaworsky et al., 1998).

Smoke condensate from cigarettes with and without the addition of ingredients to the tobacco was also evaluated for its carcinogenic potency in the mouse skin painting assay. When applied over 26 weeks to the shaved back skin of mice, tumor incidence, latency, and multiplicity were essentially the same for the cigarette types with and without the ingredient (Gaworsky, 1999).

2.2 Processed tobacco business ?

The most important types of processed tobacco are those in which reconstituted sheet or expanded tobacco technology is employed. In the first process, ground leaf material or tobacco particles that are too small to be used in cigarette filler are processed (e.g., cast sheet and papermaking process) to a reconstituted sheet material that can be used as cut filler. In the second process the original cellular structure of the tobacco leaf, which collapses to some extent during curing, is partially restored. The most important approach in this regard is the DIET (Dry Ice Expanded Tobacco) process, in which tobacco is soaked with liquid carbon dioxide and later heated up to initiate the conversion of carbon dioxide to its gas form, which enlarges the leaf material considerably (Norman, 1999).
There has been some concern that the processing might increase the biological activity of the cigarette smoke. Consequently, both types of processed tobacco have been investigated as the sole tobacco material in cigarettes and compared to cigarettes with unprocessed tobacco.

Patents

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The chemical composition of the smoke from processed tobacco is fully comparable to that from native, unprocessed tobacco, i.e., there are no new smoke components not already detectable in the smoke of cigarettes made from unprocessed tobacco. However, there are distinct differences in the yield and the composition of smoke. Depending on the sheet process, the yields of nicotine, tar, and other smoke components per cigarette might be significantly lower or higher in processed sheet cigarettes compared to control cigarettes with unprocessed tobacco. However, there is no correlation between the

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yields and the amount of smoke constituents such as benzo(a)pyrene (B(a)P) or acrolein per gram tar. Their concentration in tar might differ significantly from control cigarettes in both directions. Expanded tobacco generally yields less tar, nicotine, and other smoke constituents per cigarette. This is in line with the lower mass of tobacco in the filler. Per gram tar, however, the amounts of some constituents, e.g., acrolein and formaldehyde are even higher. (Halter and Ito, 1978)

FTC #7 1996 changing cig Hoff

The biological activity of smoke from processed tobacco has been shown not to be increased compared to that from unprocessed tobacco. Actually, it has been reported that cigarette smoke condensate from sheet material and expanded tobacco is less carcinogenic in the mouse skin painting assay than condensate from control cigarettes (Hoffmann and Wynder, 1976; US National Cancer Institute, 1980). In smoke inhalation studies with such cigarettes, a significant, reduced activity regarding laryngeal cancerous and precancerous lesions in hamsters was observed (Dontenwill, 1974). Data from a 2-year inhalation carcinogenicity study with rats exposed head-only to cigarette smoke from cigarettes with expanded and non-expanded tobacco, with one exception, did not reveal any notable statistically significant differences between sham-exposed and smoke-exposed rats with respect to tumor spectrum, tumor bearers, or tumor multiplicity. When grouped together, however, tumors in the head region and upper respiratory tract numerically higher in the smoke-exposed rats than in the sham-exposed rats, but there were no differences between the expanded and the non-expanded tobacco exposure groups (Roemer, 1998).

2.3 Natural material in or on native tobacco

Data on effects due to the actual reduction or removal of natural components of tobacco are scarce. The main reason for this might be that the removal of single target substances also affects other non-targeted substances and may have a profound effect on the taste of the resulting cigarette smoke, potentially leading to non-acceptance by the smoker. + exposure parameters

One of the most famous examples in this area is the reduction of nitrate in tobacco. The concentration of nitrate has been shown to correlate directly with the formation of nitrosamines in smoke—an effect that can be explained by nitrosation, e.g., of tobacco alkaloids (Adams et al., 1984). The nitrate concentration in tobacco filler can be modulated by direct nitrate addition to the filler, by the use of different nitrogen-fertiliz~~ed crops~~ ers, different tobacco strains (e.g., bright tobacco low in nitrate and Burley high in nitrate), or tobacco that has been nitrate reduced, e.g., ~~by bacterial denitration~~ crystal. The effect of a modulation in nitrate concentration on the smoke chemistry is different for nitrosamines and polycyclic aromatic hydrocarbons (PAHs), e.g., B(a)P. While nitrosamines increase with increasing nitrate concentration, the PAHs decrease significantly. The difference between a high nitrate Burley cultivar and a low nitrate Bright is a factor of 15 or higher for the yield of both carcinogens, but in different directions (Hoffmann and Hoffmann, 1997). As in smoke chemistry, the effect on biological activity also goes in different

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directions depending on the test system used. In the Ames assay with TA 98 as well as in a cytotoxicity assay with mammalian V79 cells, there was an increased biological activity with increased nitrate concentration (Roemer, 1990). The tumor formation in mouse skin after dermal application of smoke condensate (Wynder and Hoffmann, 1969) and induction of laryngeal cancerous and precancerous lesions after smoke inhalation (Dontenwill, 1974) were decreased with increased nitrate concentration. On the other hand, it has been unequivocally shown that most of the tobacco specific nitrosamines are potent carcinogens, some even with a specificity directed to the lungs (Hoffmann et al, 1994).

Recently a report on the partial removal of tobacco protein by water extraction and subsequent protease digestion was published. The condensate of smoke from these cigarettes showed a reduction in mutagenicity of 50 to 80 % in the Ames assay with tester strains TA98 and TA100 (Clapp et al., 1999) This is in line with the known high mutagenicity of protein and amino acid pyrolysates, pointing in the direction of heterocyclic aromatic amines as active agents (Matsumoto et. al., 1978). The data are corroborated by an increased Ames activity of cigarette smoke condensate when amino acids, especially tryptophan, are added to the tobacco (Tewes, 1992). Data on smoke chemistry, cytotoxicity, genotoxicity, general animal toxicity, and last but not least, of taste and resulting consumer acceptability of cigarettes with protein extracted tobacco have not yet been reported.

A brand of almost fully de-nicotinized cigarettes (Next ®) was marketed in 1990. However, this type of cigarette was not accepted by the consumer and consequently its production was stopped. Data on this cigarette refer only to humans and report the absence of an effect on the EEG (beta 2 and delta magnitude) and a lower increase in heart rate when compared to a control cigarette.

Only very recently approaches used to reduce the formation of tobacco-specific nitrosamines (TSNAs) by more than 90 % have been reported. In Burley tobacco a microbiological nitrosation is considered to be the primary source and seems to be prevented by microwave treatment of the tobacco that kills the bacteria (Day, 1999). For flue-cured tobacco this process seems of secondary importance. Here, N_XO_Y combustion gases of direct-fired burners in flue-cured barns appear to be mainly responsible for the formation of TSNAs. The use of heat exchangers instead of direct-fired burners thus may eliminate this primary source (Peele, Edwards, and Gentry, 1999). Further potential effects, other than the reduction of TSNAs, and especially further smoke chemistry and toxicological data have not yet been reported.

3 Correlation between Changes in Cigarette Filler and Disease Incidence in Humans

What is the evidence that the data presented in this brief commentary have any relevance for disease incidence in humans?

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This second part of Question 3 alludes to the very heart of the cigarette testing paradigm. The cigarette testing paradigm follows in essential parts the testing strategy as applied, e.g., by the pharmaceutical industry. It is based on the following assumptions:

- There are validated biological assay systems proven to be reproducibly responsive to compounds known or highly likely to be causative in the development of a human disease.
- Substances of unknown relevance to humans are considered to be most probably a causative agent for the disease under consideration when the respective assay shows a positive response.
- In the case of conflicting results of different assay systems, the assay system with the most physiological similarity to humans is considered to be of higher relevance if there are no strong arguments to speak against this assumption

So far, the above assumptions are the same as applied by other industries; however, because cigarette smoke is responsive per se in quite a lot of assays, the consequences of product changes in both a beneficial and a detrimental direction must be determined on a quantitative basis rather than a yes/no-answer. This leads to the following additional assumption.

- Cigarette smoke is a complex mixture. As that, a quantitative comparison of two smoke types in a biological assay is only valid if these smoke types, in their chemical nature, are not essentially different (an explanation for this restriction would exceed the scope of this brief commentary). If this applies, then a higher response in an assay can be taken as an indication of a higher potential to induce the respective disease in humans. Smoke or smoke fractions from cigarettes with and without a modification are considered to fulfill the prerequisite of essential similarity, consequently the quantitative approach is adequate for comparing them.

Applying these assumptions to the candidates evaluated with regard to their possible removal from the filler of cigarettes, then neither

- flavors or other ingredients nor
- processed tobacco

seem to give rise to concern. Consequently their removal is not expected to result in any benefit for the consumer.

Due to the limited amount of data it is unclear whether the removal of

- protein and
- TSNAs

in total have beneficial effects, or, in the case of protein, whether the removal leads to a product that, tastewise, can be smoked at all. In this regard, the removal of

- nicotine

has been shown not to be acceptable by the consumer.

A challenge is obviously to appraise the removal of

- nitrate

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from the tobacco with regard to its human relevance as its removal leads not only to a decrease in the carcinogenic nitrosamines, but also to an increase in carcinogenic PAHs. A concept for weighing such changes in chemical composition of complex mixtures by calculating indices for cancer- and non-cancer risks has been proposed recently (Vorhees et al., 1997). However, up to now, no one has come to any conclusion when two equally relevant assays give different answers (here tumor formation in mouse skin and hamster larynx confronted with the bulk of evidence that nitrosamines are carcinogenic). Obviously more research is needed to get an insight into the mechanisms relevant to humans .

4 Literature

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[a](data currently not yet filed) !!!!

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